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DERWENT-WEEK: 200333



TITLE: Bacteriophage-dependent method for producing biologically active proteins or peptides, involves employing an Escherichia coli transformed with a plasmid containing the targeted gene(s) operably linked to a promoter

INVENTOR: CHERNYKH, S I; KORDYUM, V A ; SLAVCHENKO, I Y ; VOZIANOV, O F
; VOZIANOV, O

PATENT-ASSIGNEE: PHAGE BIOTECHNOLOGY CORP[PHAGN] , CHERNYKH S I[CHERI],
KORDYUM V A[KORDI], SLAVCHENKO I Y[SLAVI], VOZIANOV O[VOZII]

PRIORITY-DATA: 2000US-225437P (August 15, 2000) , 2001US-0929918 (August 15,
2001)

PATENT-FAMILY:

| PUB-NO | PUB-DATE | LANGUAGE | PAGES | MAIN-IPC |
|---|---|---|---|---|
| EP 1309604 A2 | May 14, 2003 | E | 000 | C07H 021/04 |
| WO 200214468 A2 | February 21, 2002 | E | 044 | C12N 000/00 |
| AU 200184914 A | February 25, 2002 | N/A | 000 | C12N 000/00 |
| US 20020090678 A1 | July 11, 2002 | N/A | 000 | C12P 021/02 |

DESIGNATED-STATES: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK
NL PT RO SE SI TR AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ

DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL
TJ TM TR TT TZ UA UG UZ VN YU ZA ZW AT BE CH CY DE DK EA ES FI FR GB GH GM GR
IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW

APPLICATION-DATA:

| PUB-NO | APPL-DESCRIPTOR | APPL-NO | APPL-DATE |
|---|---|---|---|
| EP 1309604A2 | N/A | 2001EP-0964014 | August 15, 2001 |
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| EP 1309604A2 | Based on | WO 200214468 | N/A |
| WO 200214468A2 | N/A | 2001WO-US25477 | August 15, 2001 |
| AU 200184914A | N/A | 2001AU-0084914 | August 15, 2001 |
| AU 200184914A | Based on | WO 200214468 | N/A |
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INT-CL (IPC): C07H021/04, C12N000/00 , C12N001/21 , C12N015/74 , C12P021/00 , C12P021/02

ABSTRACTED-PUB-NO: US20020090678A

BASIC-ABSTRACT:

NOVELTY - Producing a biologically active protein comprising infecting a strain of Escherichia coli, which has been transformed with a plasmid having at least one copy of an expressible gene encoding a biologically active protein operably linked to a phage T7 polymerase promoter, with a bacteriophage capable of mediating delayed lysis, is new.

DETAILED DESCRIPTION - The method comprises:

(a) transforming a strain of E. coli with a plasmid having at least one copy of an expressible gene encoding a biologically active protein, operably linked to a T7 polymerase promoter, where the E. coli strain is capable of expressing the gene for T7 RNA polymerase;

(b) infecting the transformed bacterial host cell with a bacteriophage capable

of mediating delayed lysis; and

(c) cultivating the E. coli host cell under a culture condition that induces lytic growth of the cell without lysis until a desired level of production of the protein is reached, where the protein is produced as a soluble, biologically-active protein.

An INDEPENDENT CLAIM is also included for a chemically synthesized nucleic acid
consisting of a 630 nucleotide sequence (I) fully defined in the specification.

USE - The method is useful for the phage dependent superproduction of biologically active protein and peptides. The method is particularly useful for enhancing the production of heterologous proteins in bacterial host cells.

ABSTRACTED-PUB-NO: WO 200214468A

EQUIVALENT-ABSTRACTS:

NOVELTY - Producing a biologically active protein comprising infecting a strain of Escherichia coli, which has been transformed with a plasmid having at least one copy of an expressible gene encoding a biologically active protein operably linked to a phage T7 polymerase promoter, with a bacteriophage capable of mediating delayed lysis, is new.

DETAILED DESCRIPTION - The method comprises:

(a) transforming a strain of E. coli with a plasmid having at least one copy of an expressible gene encoding a biologically active protein, operably linked to a T7 polymerase promoter, where the E. coli strain is capable of expressing the gene for T7 RNA polymerase;

(b) infecting the transformed bacterial host cell with a bacteriophage capable of mediating delayed lysis; and

(c) cultivating the E. coli host cell under a culture condition that induces lytic growth of the cell without lysis until a desired level of production of the protein is reached, where the protein is produced as a soluble, biologically-active protein.

An INDEPENDENT CLAIM is also included for a chemically synthesized nucleic acid
consisting of a 630 nucleotide sequence (I) fully defined in the specification.

USE - The method is useful for the phage dependent superproduction of biologically active protein and peptides. The method is particularly useful for enhancing the production of heterologous proteins in bacterial host cells.

CHOSEN-DRAWING: Dwg.0/12

TITLE-TERMS: BACTERIA DEPEND METHOD PRODUCE BIOLOGICAL ACTIVE PROTEIN EMPLOY
ESCHERICHIA COLI TRANSFORM PLASMID CONTAIN GENE OPERATE LINK
PROMOTE

DERWENT-CLASS: B04 D16

CPI-CODES: B04-C01; B04-E02; B04-E08; B04-F10A3E; B04-F11; B04-N0400E;
D05-C12; D05-H08; D05-H12A; D05-H12E; D05-H14A1; D05-H17A2;

CHEMICAL-CODES:
Chemical Indexing M1 *01*
Fragmentation Code
M423 M430 M782 M905 N135 Q233
Specfic Compounds
A00NSK A00NSQ A00NSM

Chemical Indexing M1 *02*
Fragmentation Code
M423 M430 M782 M905 N135 Q233
Specfic Compounds
A012PK A012PQ A012PM

Chemical Indexing M1 *03*
Fragmentation Code
M423 M430 M782 M905 N133 N135 Q233
Specfic Compounds
A00GTK A00GTQ A00GTM

Chemical Indexing M1 *04*
Fragmentation Code
M423 M720 M905 N133 N135 Q233
Specfic Compounds
A00H1K A00H1P

Chemical Indexing M1 *05*
Fragmentation Code
M423 M720 M905 N133 N135 Q233
Specfic Compounds
A00H3K A00H3P

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(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US01/25537

(22) International Filing Date: 15 August 2001 (15.08.2001)

(25) Filing Language: English

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(30) Priority Data:
60/225,406 15 August 2000 (15.08.2000) US

(71) Applicant: **PHAGE BIOTECHNOLOGY CORPORATION** [US/US]; 14272 Franklin Avenue, Suite 100, Tustin, CA 92780 (US).

(72) Inventors: **STEGMANN, Thomas, J.**; Spiegel Strasse #10, 36100 Petersberg (DE). **KORDYUM, Vitaliy, A.**; Artyoma Street 53, Kiev-053, 254053 Ukraine (UA). **CHERNYKH, Svitlana, L**; Lomonosova Street 29, Apt. 6, Kiev-127, 252127 Ukraine (UA). **SLAVCHENKO, Iryna, Yu.**; Revutskogo Street 44, Apt. 81, Kiev-068, 253068 Ukraine (UA). **VOZIANOV, Oleksandr, F.**; Desyatinnaya Street 10, Apt. 6, Kiev-025, 252025 Ukraine (UA).

(74) Agent: **ALTMAN, Daniel, E.**; KNOBBE, MARTENS, OLSON & BEAR, LLP, 620 Newport Center Drive, Sixteenth Floor, Newport Beach, CA 92660 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EC, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: A METHOD OF PRODUCING BIOLOGICALLY ACTIVE HUMAN ACIDIC FIBROBLAST GROWTH FACTOR AND ITS USE IN PROMOTING ANGIOGENESIS

(57) Abstract: The gene of human acidic fibroblast growth factor 155 (haFGF 155) has been obtained by chemical synthesis. The nucleotide sequence of haFGF 155 gene has been deduced on the basis of haFGF 155 amino acid sequence as described in the literature. The amino acid sequence of the synthesized haFGF 155 does not differ from those described in the literature. The nucleotide sequence of haFGF gene differs from those described previously. For chemical synthesis of haFGF 155 gene, codons were used which are the ones most often used by E. coli in highly expressed E. coli proteins. A plasmid with haFGF 155 (phaFGF 155) gene was obtained and was used to transform E. coli. Production of haFGF 154 protein was achieved by cultivation of the producer strain under conditions which slow down the lytic development of lambda phage. The haFGF 154 protein accumulated in culture medium in a soluble condition as a result of the producer strain cells lysis by the lambda phage. The haFGF 154 protein constituted 20% of the soluble protein accumulated in the culture medium and its biological activity was demonstrated by its ability to generate new vessels (angiogenesis). The initiator methionine residue at position 1 of the FGF 155 protein was completely removed during protein synthesis resulting in an FGF 154 amino acid product. The use of the phage-dependent method to produce other forms of the haFGF protein is also disclosed.

WO 02/14471 A2

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DIALOG(R)File 155:MEDLINE(R)


07009372 91250092 PMID: 1828240

Cloning of xylanase gene of Streptomyces flavogriseus in Escherichia coli and bacteriophage lambda-induced lysis for the release of cloned enzyme.

Srivastava R; Ali S S; Srivastava B S

Division of Microbial Genetics, Central Drug Research Institute, Lucknow, India.

FEMS microbiology letters (NETHERLANDS) Mar 1 1991, 62 (2-3) p201-5, ISSN 0378-1097 Journal Code: 7705721

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The xylanase gene of Streptomyces flavogriseus was cloned in pUC8 plasmid and expressed in Escherichia coli lysogenic for lambda cI857. lambda-Induced lysis of E. coli at 42 degrees C allowed efficient release of cloned enzyme activity in extracellular environment. The xylanase gene was located in the 0.8-kb HindIII fragment and coded for 18,000 Mr xylanase.

same entity

US006268178B1

(12) **United States Patent**
**Kordyum et al.**

(10) **Patent No.: US 6,268,178 B1**
(45) **Date of Patent: Jul. 31, 2001**

(54) **PHAGE-DEPENDENT SUPER-PRODUCTION OF BIOLOGICALLY ACTIVE PROTEIN AND PEPTIDES**

(75) Inventors: **Vitaliy A. Kordyum; Svetlana I. Chernykh; Irina Y. Slavchenko; Oleksandr F. Vozianov**, all of Kiev (UA)

(73) Assignee: **Phage Biotechnology Corp.**, Irvine, CA (US)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/318,288**

(22) Filed: **May 25, 1999**

(51) **Int. Cl.**[7] ..... **C12P 21/00**; C12N 7/00; C12N 1/21; C12N 15/70; C07H 21/04

(52) **U.S. Cl.** ..... **435/69.1**; 435/69.51; 435/320.1; 435/252.3; 435/471; 435/235.1; 536/23.1

(58) **Field of Search** ..... 435/69.1, 252.3, 435/69.4, 69.51, 243, 320.1, 235.1, 471; 536/23.1

(56) **References Cited**

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*Primary Examiner*—David Guzo
*Assistant Examiner*—Gerald G. Leffers, Jr.
(74) *Attorney, Agent, or Firm*—Knobbe, Martens, Olson & Bear, LLP

(57) **ABSTRACT**

This invention relates to a method for enhancing the production of biologically active proteins and peptides in bacterial cells by infecting bacterial cells of the producer strain, which contain a plasmid with one or more targeted genes, with bacteriophage λ with or without the targeted gene(s). The phage increases synthesis of the targeted protein and induces lysis of the producer strain cells. Super-production is achieved by cultivating the producer strain cells under culture conditions that delay lytic development of the phage. The biologically active proteins and peptides subsequently accumulate in a soluble form in the culture medium as the cells of the producer strain are lysed by the phage.

**16 Claims, No Drawings**

Same entity

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(30) Priority Data:
09/318,288 25 May 1999 (25.05.1999) US

(71) Applicant: **PHAGE BIOTECHNOLOGY CORPORATION** [US/US]; 18300 Von Karman Avenue, Suite 440, Irvine, CA 92612 (US).

(72) Inventors: **KORDYUM, Vitaliy, A.**; Artyoma St. 53, Apartment 33, 254053 Kiev-053 (UA). **CHERNYKH, Svetlana, I.**; Lomonosova St. 29, Apartment 6, 252127 Kiev-127 (UA). **SLAVCHENKO, Irina, Y.**; Revutskogo St. 44, Apartment 81, 253068 Kiev-068 (UA). **VOZIANOV, Oleksandr, F.**; Desyatinnaya St. 10, Apartment 6, 252025 Kiev-025 (UA).

(74) Agent: **SIMPSON, Andrew, H.**; Knobbe, Martens, Olson and Bear, LLP, 620 Newport Center Drive, 16th floor, Newport Beach, CA 92660 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 00/71731 A1

(54) Title: PHAGE-DEPENDENT SUPER-PRODUCTION OF BIOLOGICALLY ACTIVE PROTEIN AND PEPTIDES

(57) Abstract: This invention relates to a method for enhancing the production of soluble and biologically active proteins and peptides in bacterial cells by infecting bacterial cells of the producer strain, which contain a plasmid with one or more targeted genes, with bacteriophage λ with or without the targeted gene(s). The phage increases synthesis of the targeted protein and induces lysis of the producer strain cells. Super-production is achieved by cultivating the producer strain cells under culture conditions that delay lytic development of the phage. The biologically active proteins and peptides subsequently accumulate in a soluble form in the culture medium as the cells of the producer strain are lysed by the phage.